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TITLE: Development of Novel Combinatorial Treatment to Prevent
Chemotherapeutic Resistance and Enhance Efficacy of Riluzole in a Rodent Model
of SCI

PRINCIPAL INVESTIGATOR: Raymond Grill

CONTRACTING ORGANIZATION:
University of Mississippi Medical Center
Jackson, MS 39216-4505

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14. ABSTRACT Overall goal of this proposal is to use a pharmacological approach to prevent or significantly reduce the onset of chemotherapeutic resistance that we have recently observed/described following spinal cord injury (SCI). In our initial, published report, we found that SCI produced a sustained upregulation of P-glycoprotein (Pgp) within the spinal cord that prevented access of systemically (intraperitoneally) administered riluzole. This chemotherapeutic resistance was found to be permanent within the spinal cord as we continued to detect elevated Pgp at the lesion site as well as in the cervical and lumbar cords out to at least 10 months post-injury. While our rodent study was ongoing, a multi-center clinical trial was performed assessing riluzole as an acute treatment for SCI. While showing a trend toward improved function, the results were not statistically significant. Pharmacokinetic assessment of orally-riluzole bioavailability, however, showed a dramatic reduction of plasma concentrations of riluzole between 3 and 14 days of treatment. Based on our rodent spinal cord data, we asked whether this could suggest a Pgp-dependent reduction of orally-administered riluzole. We subsequently showed (in preliminary data for this project) that SCI produced a rapid induction of Pgp protein expression in the gastrointestinal tract of rats. This lead us to hypothesize that: 1) SCI produces systemic chemotherapeutic resistance, 2) that targeting the inflammatory pathways that promote Pgp induction will prevent onset of chemotherapeutic resistance, 3) that co-administration of riluzole with the anti-inflammatory treatment will both suppress GI Pgp and enhance plasma concentrations of riluzole, and 4) that this combinatorial treatment will lead to a preservation of motor function and an attenuation in long-term pathologies like neuropathic pain in rats following an acute therapeutic intervention. Within this first year, our efforts have focused on Aim 1a (generating a surgical time course in which we are collecting GI and spinal tissues for the assessment of Pgp expression). We have also been working with our Co-Investigator, Stanley Smith, Ph.D., to optimize the LC/MS technique to measure plasma and spinal bioavailability of orally-administered riluzole.					
15. SUBJECT TERMS spinal cord injury (SCI), gastrointestinal (GI), P-glycoprotein (Pgp), chemotherapeutic resistance, mass spectrometry, riluzole, licofelone, neuropathic pain, locomotor, bioavailability					
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Introduction:

The overall goal of this DOD-funded proposal is to target the pathological process known as chemotherapeutic resistance in spinal cord injury (SCI) in order to enhance the bioavailability and efficacy of riluzole, an FDA-approved neuroprotective drug. In a previously published study, we demonstrated that SCI resulted in the upregulation of P-glycoprotein (Pgp), an energy driven pump that sequesters and limits the amount of a wide range of substances, both endogenous as well as exogenous (such as drugs) from tissues. Pgp is a significant contributor to the process of chemotherapeutic resistance in many forms of cancer that blocks access of systemically-administered drugs into tumors, reducing their therapeutic efficacy. As there have been no pharmacological treatments shown to be effective in the clinic for the treatment of SCI, we hypothesized that spinal trauma may establish chemotherapeutic resistance in a manner that is analogous to what is observed in cancer. We demonstrated/published these results showing that riluzole, an FDA-approved drug that has been under evaluation in the clinic for acute treatment of SCI, was excluded from the spinal cords of injured rats. We also demonstrated that this effect was due to an SCI-dependent induction of Pgp within the traumatized spinal cord. Finally, we further demonstrated that if we targeted inflammatory conditions using a novel, dual inhibitor of both cyclooxygenase AND 5-lipoxygenase (licofelone) we could prevent the SCI-dependent induction of Pgp. If we then co-administered licofelone with riluzole, this dramatically enhanced the intraspinal bioavailability of riluzole. As mentioned above, riluzole was undergoing a clinical trial as an early intervention for SCI. While showing a strong trend towards efficacy, the results were not significant. Of significant interest to us though, a pharmacokinetic analysis from the clinical trial showed that plasma concentrations of orally-administered riluzole plummet between 3-14 days of treatment. When we examined Pgp expression within the gastrointestinal tract, the sole route through which orally-administered riluzole would gain access to the plasma, we found a more than two-fold induction of Pgp. Thus, the primary goal of this study is to determine whether orally-administered licofelone can suppress SCI-dependent increases in GI and spinal Pgp expression and enhance the systemic and intraspinal bioavailability and efficacy of riluzole. We are measuring the time-dependent induction of Pgp after SCI under vehicle vs. licofelone-treatment (Aim 1a) and assessing the effects of co-administered riluzole bioavailability in plasma and spinal cord (Aim 1b). The second aim will explore the effects of this novel combinatorial treatment on both locomotor and neurosensory function. During this first year we have focused on Aim 1a and are optimizing the conditions for Aim 1b (see below).

Keywords:

Spinal cord injury (SCI), chemotherapeutic resistance, P-glycoprotein (Pgp), gastrointestinal (GI), riluzole, licofelone, inflammation, anti-inflammatory, pharmacokinetics, plasma

Accomplishments:

During this first year, we have mainly been focused on generating the initial longitudinal time course for the measurement of Pgp within the spinal cords and GI tissues following SCI. This involves generating the following groups:

- 1) Naïve (uninjured control group for establishing baseline conditions) (N=8 for only a single time point)
- 2) SCI+1 mg/kg licofelone
- 3) SCI+10 mg/kg licofelone
- 4) SCI+100 mg/kg licofelone
- 5) SCI+vehicle

N=8/group/time point, but with groups being harvested at 1, 2, 3 and 7 days post-SCI. This requires the 8 naïve animals listed above with 4 groups X 8 rats/group X 4 time points= 128 rats. We are more than half way through the acquisition of these animals at this point. We initially encountered two problems (see below for greater detail) that slowed our progress, but both have been addressed and we are proceeding with the originally-anticipated speed.

While focusing on Aim 1a, we have also been working with our Co-Investigator, Dr. Stanley Smith, from the

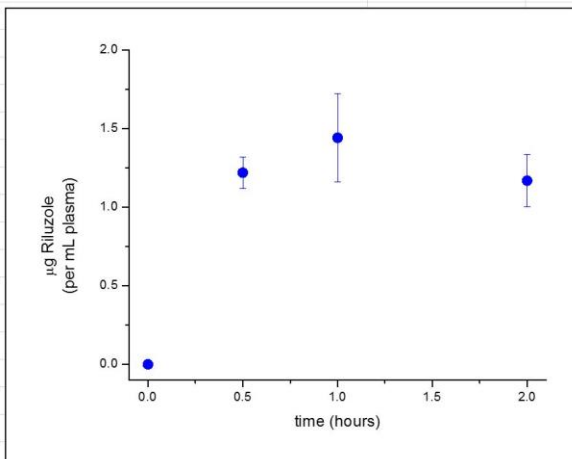


Fig. 1: We detect a rapid infiltration of orally-administered riluzole into the plasma by 30 minutes post-delivery using mass spectrometry. Peak concentrations appear to occur at 1 hour with a gradual reduction observed by 2 hours post-delivery.

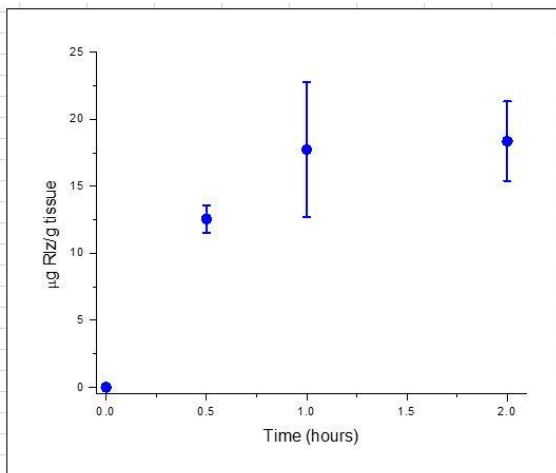


Fig. 2: We detect a similar time-dependent increase in intra-spinal riluzole bioavailability in uninjured, orally-treated subjects using mass spectrometry.

Department of Pharmacology and Toxicology at UMMC to optimize our ability to detect orally-administered riluzole in both the plasma as well as spinal cords of rats. Dr. Smith has been working with Ms. Suzanne Sereduck (Research Assistant in Dr. Grill's laboratory) to optimize collection, extraction and detection methodologies. This led to an initial assessment of both plasma and spinal concentrations of orally-administered riluzole (10 mg/kg) given to naïve, uninjured male Sprague-Dawley rats via a one-time oral gavage delivery. Riluzole was given to cohorts of these rats (N=5/cohort) with subjects subsequently sacrificed at 30 minutes, 1 and 2 hours post-riluzole delivery. Control rats were given the vehicle but no riluzole. At

the time of sacrifice, subjects were euthanized and decapitated with blood and the spinal cords (2 centimeters of thoracic cord) collected. Blood was separated into plasma and

frozen while spinal cords were frozen immediately following removal. Drs. Smith and Ms. Sereduck extracted the riluzole and performed a mass spectrometry analysis on both the plasma and cord samples that demonstrated a time dependent presence increase and subsequent decrease of plasma riluzole (Fig. 1). In addition, spinal cord tissues from these same animals were thawed, extracted and run on a mass spectrometer in Dr. Smith's Biochemistry Core. In uninjured spinal tissue we observe detectable concentrations of riluzole at 30 minutes post-oral gavage delivery (Fig. 2). Intraspinal riluzole concentration appears to peak between 1 and 2 hours. While we are prepared now to perform the

plasma/bioavailability analyses outlined in Aim 1b, we first wish to optimize the procedure for measuring

riluzole concentrations within the feces of naïve vs. injured rats. Based on the data generated both by the human clinical trial as well as our subsequent detection of an SCI-dependent increase in GI Pgp expression, we hypothesized that following SCI, riluzole provided via the oral route (as was performed in the human clinical trial) experiences reduced transit through the wall of the GI tract. As a result, orally-administered riluzole should exhibit an increase in concentration within the feces due to poor GI absorption. We are currently working out the parameters for measuring riluzole concentration within the feces using mass spectrometry vs. HPLC-based detection methods.

Summary: We continue to collect the tissues required for Pgp assessment in Aim 1a. We are waiting until completion of acquisition before starting the Pgp Western blot analyses in GI and spinal tissues. We are now prepared, however, to begin Aim 1b (assessment of riluzole bioavailability in plasma and spinal cord in naïve vs. injured (vehicle treated) vs. licofelone-treated subjects).

Training/Personal Development:

I was recently approached by Dr. Christopher Seward, a third year neurosurgical resident at UMMC. Dr. Seward expressed an interest in performing a research block in my laboratory. I agreed and have trained him in the spinal contusion injury model. Dr. Seward is now working extensively on the remaining surgeries needed for Aim 1a. As he is a trained neurosurgeon, this has dramatically increased the movement of this aim. I am including him in the molecular analyses as well and will include him on subsequent publications.

Results Disseminated:

No major results to report at this stage.

Plans for next reporting period:

Continue with surgeries and tissue collections. Optimize riluzole detection techniques from collected fecal samples.

Impact:

Nothing to report as of yet

Changes/Problems:

We encountered two significant problems during the first year of the study. Both problems, however, arose from the same issue. During the summer of 2016, my Research Assistant/Lab Manager left the lab with short notice to go to medical school. I experienced a several month slowdown as all project efforts fell to me. In September of 2016, I hired Ms. Suzanne Sereduck to assist with Western blot and biochemical preparation/analysis of riluzole bioavailability. Also, as mentioned above, I have recently added Dr. Christopher Seward to the study to assist with animal surgeries and tissue collection. This is dramatically enhancing our productivity. Also, Dr. Seward's addition will not impact the budget of the proposal as his salary is provided by his department through protected research time.

Products:

Nothing to report

Participants:

1) Raymond J. Grill, Ph.D.

PI

ORCID ID: unsure of this

Nearest person month: 1.8 mo

Contribution to Project: 1) General oversight, 2) surgical procedures and post-operative care, 3) providing training on surgical procedures and post-operative care to Research Assistant and Neurosurgical Resident.

Funding support: This grant

2) Stanley Smith, Ph.D.

Co-Investigator

Nearest Person Month: 1.2

Contributions to Project: Establishing detection protocols for measuring riluzole concentrations in plasma and spinal cord using mass spectrometry.

Funding Support: This grant

3) Ms. Suzanne Sereduck (MA)

Research Assistant III

Nearest Person Month: 12

Contributions to project: 1) post-operative care, 2) assisting in tissue collection, 3) sample preparation for mass spectrometry analysis.

Funding Support: This grant

4) Christopher Seward, MD

Neurosurgical Resident

Nearest Person Month: N/A

Contributions to project: 1) performing spinal contusion surgery, 2) assisting in tissue collection

Funding Support: University of Mississippi Medical Center, Department of Neurosurgery

Development of novel combinatorial treatment to prevent chemotherapeutic resistance and enhance efficacy of riluzole in a rodent model of SCI.

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PI: Raymond J. Grill, Ph.D.

Org: University of Mississippi Medical Center

Award Amount: \$479,697

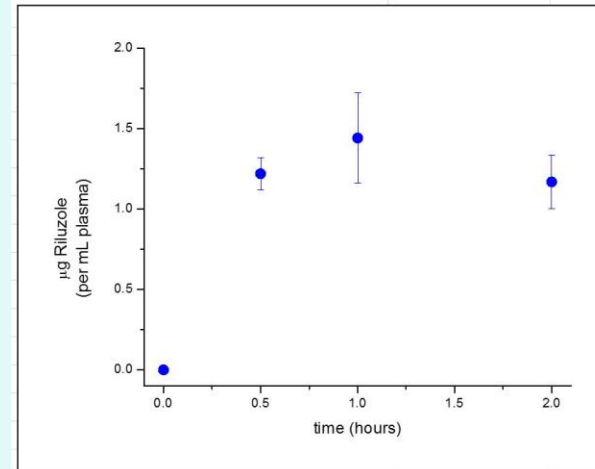


Study/Product Aim(s)

- Aim 1a: Perform longitudinal assessment of the induction of Pgp in the gastrointestinal tract of the adult male rat over 7 days post-SCI
- Aim 1b: Perform bioavailability analysis of orally-administered riluzole in both plasma and spinal cord samples in naïve vs. injured rats following vehicle or licofelone treatment. Goal is to determine whether suppression of GI Pgp expression with licofelone will enhance systemic and spinal riluzole bioavailability
- Aim 2: Determine whether combined riluzole with licofelone enhances locomotor recovery and prevents onset of neuropathic pain following SCI.

Approach

Overall procedures involve use of clinically-relevant rat spinal contusion injury. Outcome measures include assessment of Pgp protein expression in spinal cord and GI tissues and riluzole bioavailability in plasma and cord using mass spect.



Accomplishment: we have optimized the mass spectrometry methodology for measuring riluzole concentrations in both plasma and spinal cord samples.

Timeline and Cost

Activities	CY	15-16	17		
Aim 1a: Explore longitudinal induction of Pgp in GI and spinal tissues					
Aim 1b: explore bioavailability of riluzole in vehicle vs. licofelone treated rats after SCI					
Text (Major aim/study/milestone)					
Estimated Budget (\$K)		\$000	\$000	\$000	\$000

Goals/Milestones (Example)

CY15-16 Goals – Perform all surgeries needed for aims 1a,b

☒ Functionality tests of integrated firmware and software

CY17 Goals – complete Aims 1a,b with Pgp quantification and riluzole bioavailability measurements

Comments/Challenges/Issues/Concerns

- performance slowed due to loss of personnel
- Situation resolved with addition of new lab manager and surgeon.

Budget Expenditure to Date

Projected Expenditure:

Actual Expenditure:

Updated: 12/15/16